



## CLINICAL OBSERVATION

## Antithymocyte globulin/antilymphocyte globulin plus kidney-nourishing Chinese Medicinal: effect on severe aplastic anemia

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### Abstract

**OBJECTIVE:** To explore the effect of antithymocyte globulin (ATG)/antilymphocyte globulin (ALG) plus kidney-nourishing Chinese medicinal (KNCM) on severe aplastic anemia (SAA).

**METHODS:** Twenty-five subjects of severe aplastic anemia were treated with ATG/ALD plus KNCM between 1992 and 2009, and the clinical data before and after treatment were collected and analyzed.

**RESULTS:** Of the 25 patients, 9 were nearly cured, 6 were improved, 5 were in remission, and 5 failed. The overall effective rate was 80.0%. The 3-year, 5-year, 10-year, 15-year survival rate were respectively 98.6%, 97.3%, 97.3%, 67.5%, and median survival time was 180 months. Compared to the conditions before administering the medication of ATG/ALG plus KNCM, after 2 weeks, reticulocyte was first improved ( $P=0.001$ ); one month later, followed by palette ( $P=0.037$ ); two months later, by neutrophil cell in peripheral blood ( $P=0.001$ ); three months later, then by the hemoglobin ( $P=0.012$ ). By conducting 1-year follow-up, 1 case of complication--parox-

ysmal nocturnal hemoglobinuria (PNH) was identified and the patient still alive today.

**CONCLUSION:** ATG/ALG plus KNCM had better effect on SAA and could improve patients' survival rate.

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**Key words:** Anemia, aplastic; Reinforcing kidney; Drugs, Chinese herbal; Antilymphocyte serum; Antithymocyte serum

### INTRODUCTION

Severe aplastic anemia (SAA) is a condition that may acutely develop and have a relatively high mortality rate. It is a refractory illness of hematological diseases. Studies showed that most of aplastic anemia was caused by immune disorder.<sup>1</sup> The very severe aplastic anemia (VSAA), a much more serious condition, is characterized by higher death rate and often accompanied by heavy infection and internal hemorrhage. 20 years ago, the mortality rate of VSAA was nearly 100%. Based on VSAA's nature of acute onset, rapid development, progressive anemia, high fever and severe hemorrhage, it was defined and discussed as "Ji lao", "Re Lao" or "Xue Zheng" in Traditional Chinese Medicine (TCM). In 2004, Hematological Committee of Chinese Association of the Integration of Traditional and Western Medicine defined SAA as "Ji Sui Lao" in terms of TCM theory. The department of Xiyuan Hospital have been engaged in the research into AA and it has treated the illness with integration of TCM and Western Medicines since 1950s<sup>2-3</sup> and proposed the kidney-nourishing as the primary principle in AA's treatment with TCM, which has been acknowledged as a better scheme for chronic aplastic anemia. Starting from

1990s, antithymocyte globulin (ATG)/antilymphocyte globulin (ALG) plus kidney-nourishing Chinese medicinal (KNCM) has been used as the first line treatment for SAA and VSAA. Up to date, few reports have been published on VSAA's treatment with ATG/ALG plus KNCM. In this paper, we report our results of treating VSAA with the integration of TCM with Western Medicine from 1992 to 2009.

## METHODS

### Subjects

Twenty-seven subjects of type-I VSAA were recruited by the Department of Hematology of Xiyuan Hospital of Chinese Academy of Chinese Medicine Sciences. 2 of them dropped out after ATG administered for 2 months due to financial issue. 25 subjects, 17 males and 8 females, were treated and evaluated. Their ages ranged from 7 to 61 years with median age of 29, and the disease courses ranged from 1 to 6 months with median course of 1.1 months. 11 cases were treated with ATG (of them, 5 with horse anti-human antibody; 6 with rabbit anti-human antibody) and 14 cases were treated with ALG.

### Diagnostic criteria<sup>4</sup>

VSAA was diagnosed with Camitta's criteria (1925) for SAA plus neutrophil count which was less than  $0.2 \times 10^9/L$ .

### Treatment

Administration of ATG/ALG: ALG (swine anti-human T cell immunoglobulin, Wuhan Institute of biological Products, P. R. China), intravenously guttae (iv gtt) 20-30 mg/(kg · d). ATG (horse anti-human T cell immunoglobulin, Genzyme Polyclonals S.A.S), iv gtt 8.3-13.4 mg/(kg · d); ATG (rabbit anti-human T cell immunoglobulin, Genzyme Polyclonals S.A.S), iv gtt 2.5-3.2 mg/(kg · d), ATG (rabbit anti-human T cell immunoglobulin, Fresenius Biotech GmbH), iv gtt 5-7 mg/(kg · d). After hypersensitive tests of the subjects were confirmed negative, ATG/ALG were administered, iv gtt QD, to them for 5 days. Every time prior to the administration of ATG/ALG, 5 mg of Dexamethasone was administered by venous infusion and 25 mg of promethazine hydrochloride by intramuscular injection. Administration of KNCM: In terms of TCM theory, kidney deficiency can be classified as kidney-*Yang* deficiency and kidney-*Yin* deficiency. While the kidney was nourished, spleen might be nourished and blood activated, related toxic elements eliminated depending on the outcome of pattern differentiation.

The commonly used Chinese medicinal were as follows: Radix Rehmannia, Fructus Corni, Polugonium Multiflorum Thunb, Fructus Psoralea, Cuscuta Chinensis Lam, Radix morindae officinalis, Songaria Cynomorium and Herba Epimedii.

Addition of extra medicinal was dependent on the outcome of pattern differentiation: for the pattern with manifest kidney deficiency, Fructus mori, Glossy privet fruit, Manyflower solomonseal rhizome and Barbury wolfberry fruit were added; for the pattern with manifest kidney-*Yang* deficiency, Rhizoma curculiginis and Herba cistanches were added. For the pattern accompanied by symptoms of spleen deficiency, Radix pseudostellariae, Largehead atractylodes rhizome, Common yam rhizome was added; for the pattern accompanied by the symptoms of blood deficiency, Astragalus membranaceus, Chinese angelica, Chinese peony and Placenta hominis were added. For the pattern accompanied by blood stasis, Radix salviae miltiorrhiza, Milletia dielsiana, Leonurus heterophyllus and Rhizoma ligustici chuanxiong were added; for the pattern accompanied by heat toxin, Smilax glabra, Taraxacum, Flos lonicerae and Weeping forsythia fruit were added. The medicinal were decocted and taken twice a day in the morning and evening respectively. The course of therapy was 6 months, and two consecutive courses were administered. After finishing the two courses, all patients who showed progression were required to be treated with the same regimen for at least 1 year or more.

Supportive and palliative treatment: All patients that underwent above-mentioned treatment were orally administered Cyclosporin A. It was initially taken at the dose of 6 mg/(kg · d) BID, then the dose was modified in terms of blood drug concentration (to keep its trough concentration at 200-400 ng/mL). The treatment course was 6 month or more. After the hemogram was stable, the dose was slowly reduced and finally it ceased to be administered; Stanazolol (6-12 mg/d) or testosterone undecanoate capsule (120- 240 mg/d) was taken orally BID. After the peripheral hemogram became normal, the dose of medicine was reduced gradually and maintained for 3 years. Granulocyte-colony stimulating factor was administered at the dose of 5 µg/(kg · d) until the count of neutrophilic granulocytes was greater than  $1 \times 10^9/L$ ; Blood transfusion was performed when hemoglobin was below 60 g/L and the count of platelets was less than  $10 \times 10^9/L$  or when there was a increasing tendency of hemorrhage, the machine-collected pallet was transfused.

Prevention and treatment of adverse reaction: When ATG/ALG was used, patients were requested to stay in the wards with 100-grade air cleanliness to be kept away from infection. Their blood cell counts were examined every other day or daily when necessary. Depending on outcome of the hemogram, bone marrow might be re-examined. Hypersensitiveness and serum disease were monitored during the administration of ATG/ALG. The symptoms watched were hypertrichosis, digestical tract disorders, hand tremor, periphery sensation decreasing, pyrexia, bleeding, skin itch, myosalgia, arthrocele. Regularly hepatic and renal functions were examined, electrocardiogram and X-ray were performed, hemoclature was conducted, and chest CT performed if necessary.

Prevention and treatment of serum sickness: After ATG/ALG treatment, Prednisone acetate was orally administered at the dose of 50 mg/d. If serum sickness reaction occurred, prednisone acetate was replaced with methylprednisolone, which was administered at the dose of 40-200 mg/d, iv gtt for no more than one week, then its dose was gradually reduced. Once infection occurred, antibiotics were used. If hepatic function impairment occurred, Polyene Phosphatidylcholine and diammonium glycyrrhizinate were administered for liver protection purpose.

### Effect evaluation

The efficacy evaluation criteria was formulated based on the evaluation standard formulated by the Fourth National conference on Aplastic Anemia held in 1987.<sup>5</sup> The efficacy was graded as cure, remittance, improvement and failure. And the first 3 grades, cure, remittance and improvement, were considered having effect.

### Data analysis

SPSS 13.0 was employed for data analysis. Matched-pair sample *t*-test was performed to compare means before and after the treatment in the same group. The difference was considered significant if *P*-value was less than or equal to 0.05.

## RESULTS

### Effectiveness ATG/ALG plus KNCM in the treatment of VSAA (Table 1)

Table 1 summarizes the information of the 25 VSAA patients treated with the therapy and followed up for 1 year or more after they finished the intervention.

### The change of hemograms before and after the treatment of ATG/ALG plus KNCM (Table 2)

From Table 1, we can see that 2 months after the treat-

ment, the neutrophil started to rise significantly ( $P=0.001$ ); that hemoglobin increased manifestly 3 months after treatment ( $P=0.012$ ); that reticulocyte began to go up quickly 2 weeks after the treatment ( $P=0.001$ ); that platelet rose considerably 1 month after the treatment ( $P=0.037$ ).

### Survival Curve for the treatment of ATG/ALG plus KNCM (Figure 1)

From Figure 1, we can see that, after the Treatment of ATG/ALG plus KNCM, the survival rate for 3 years, 5 years, 10 years and 15 years were 98.6%, 97.3%, 97.3%, 67.5% respectively.

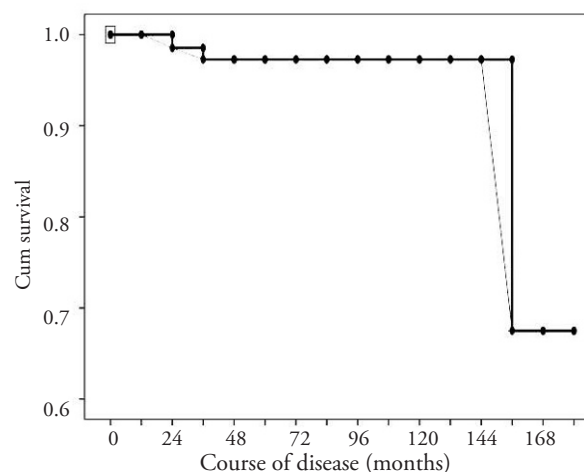


Figure 1 Survival curve for the treatment of ATG/ALG plus KNCM

### Adverse reaction and long-term complication

Allergic response occurred in 23 VSAA cases after ATG/ALG administered. Of all patients of serum sickness reaction; pyrexia occurred in 22 patients; skin rash in 21 patients; arthrodynia in 19 patients; and chest distress in 1 patient. After administered methylprednisolone, proazamine and calcium gluconate, all symptoms were improved. The serum sickness reaction oc-

Table 1 Effectiveness of ATG/ALG plus KNCM

	Cure	Remittance	Improvement	Failure	Effective rate
VSAA	9/25 (36.0%)	5/25 (20.0%)	6/25 (24.0%)	5/25 (20.0%)	20/25 (80.0%)

Notes: ATG/ALG: antithymocyte globulin/antilymphocyte globulin; KNCM: kidney-nourishing Chinese medicinal; VSAA: very severe aplastic anemia.

Table 2 Comparison of blood cell counts before and after treatment ( $\bar{x} \pm s$ )

Time point	Neutrophil ( $\times 10^9/L$ )	Hemoglobin (G/L)	Reticulocyte ( $\times 10^9/L$ )	Platelet ( $\times 10^9/L$ )
Before Treatment	0.10 $\pm$ 0.09	68.48 $\pm$ 18.10	1.24 $\pm$ 3.24	14.89 $\pm$ 14.76
1 week after treatment	0.15 $\pm$ 0.12	70.11 $\pm$ 8.28	5.47 $\pm$ 3.55	23.44 $\pm$ 15.77
2 weeks after treatment	0.37 $\pm$ 0.35	76.20 $\pm$ 16.18	7.21 $\pm$ 4.28 <sup>a</sup>	22.67 $\pm$ 12.78
1 month after treatment	0.44 $\pm$ 0.40	74.20 $\pm$ 13.61	10.11 $\pm$ 8.27 <sup>a</sup>	30.22 $\pm$ 28.76 <sup>b</sup>
2 months after treatment	1.73 $\pm$ 0.88 <sup>a</sup>	77.50 $\pm$ 42.19	13.98 $\pm$ 10.54 <sup>a</sup>	25.5 $\pm$ 21.50 <sup>b</sup>
3 months after treatment	1.65 $\pm$ 0.55 <sup>a</sup>	79.11 $\pm$ 7.36 <sup>b</sup>	15.88 $\pm$ 12.71 <sup>a</sup>	29.0 $\pm$ 16.99 <sup>b</sup>
6 months after treatment	1.73 $\pm$ 1.35 <sup>a</sup>	82.44 $\pm$ 9.40 <sup>b</sup>	18.22 $\pm$ 10.84 <sup>a</sup>	44.60 $\pm$ 40.2 <sup>a</sup>

Notes: Compared with those before treatment, <sup>b</sup>  $P < 0.05$ , <sup>a</sup>  $P < 0.01$ .

curred as early as the first day after ATG/ALG was administered and the latest one occurred on the 30th day after ATG/ALG was given and the reaction lasted from 4-8h. The time period of follow-up ranged from 51 to 172 months. The median follow-up time was 104 months. During the follow-up period, patients 2 were reported death. One relapsed and developed into PNH, who is still alive today.

## DISCUSSION

VSAA is a life-threatening disorder that the function of bone marrow is impaired. The condition leads to red marrow replaced by yellow marrow which is specialized fat cells, and the decrease of the whole blood cells. In many cases, the etiology is considered to be idiopathic, but one known cause is an autoimmune disorder in which T lymphocytes attack the bone marrow.<sup>6</sup> Aplastic anemia is sometimes associated with exposure to toxins such as benzene, or with the use of certain drugs, including chloramphenicol and carbamazepine. Exposure to ionizing radiation from radioactive materials or radiation-producing devices is also associated with the development of VSAA.

There are few records of VSAA in the ancient literatures. However, the Huangdi's Internal Classic has many descriptions of grouping symptoms such as pale, bleeding and pyrexia. In Han Dynasty, Zhongjing Zhang first coined the word of Xulao for the condition in Synopsis of Golden Chamber. In terms of TCM theory, VSAA is a long-term course illness characterized by the deficiency pattern staying throughout the whole course, which involves the deficiency of heart, liver, spleen, lung and kidney. *Qi* and blood deficiency appeared first, then kidney deficiency, and essence depletion follows. Although the condition involves heart, liver, spleen and kidney, kidney has the closest relation with VSAA. Kidney deficiency is the key factor underlying the onset and development of VSAA.

Currently, in China, the primary treatment for VSAA is intensive immunosuppressive therapy (IST) such as ATG/ALG and CsA. The fatality rate in early stage fell to 9.6%. According to some early literature, the efficiency rate of treating SAA with horse ATG only was 40%-50% and that with the combination of ATG and CsA was 70% in the treatment period between 3 and 6 months. The advantage for the combination of the medications is that ATG and CsA can act on different part of immune system. Today, ATG/CsA is still used in the regular treatment scheme for VSAA. Large number cases study in multi-centers proved that the effective rate of the treatment scheme was 70%<sup>7</sup> with 5-year survival rate up to 80%-90%.<sup>8</sup> The research into the treatment of VSAA with kidney-nourishing as principle method for AA started in 1960s. That the treatment of AA should focus on kidney was first advocated by the Hematological Committee of Chinese Association of the Integration of Traditional and Western Med-

icine on the National Conference on Blood Disease Treated by intetration of Chinese and Western Medicines held in 1986 in Dalian, China. In terms of TCM pattern differentiation for treatment, kidney deficiency patterns for AA were classified into three: *Yang* deficiency, *Yin* deficiency, both *Yang* and *Yin* deficiency. Ever since the beginning of 1990s, combination of KNCM and ATG/ALG for VSAA treatment was first used in Xiyuan Hospital in Beijing, China. In our experience of treating 25 VSAA patients, the total effective rate was 80.0%. The survival rate for 3 years, 5 years, 10 years and 15 years were 98.6%, 97.3%, 97.3%, 67.5% respectively. The reticulocyte, platelet, neutrophil and hemoglobin started to rise 2 weeks, 1 months, 2 months and 3 months after the treatment respectively. The results suggested that kidney-nourishing medicinal played a vital role in the VSAA treatment and patients' survival time.

For human body, ATG/ALG is a kind of heterogenous protein, it mainly composes of IgG, which underlie the allergy and serum sickness. Our study found that the symptoms could disappear within 2-4 days of all patients experienced allergy (23/25, 92.0%), especially 2-8h after ATG/ALG first used, by slowing down iv-gtt speed of ATG/ALG or stopping using it and taking medication such as dexamethasone. Serum sickness is a systemic allergic disease caused by blood circulation of allergen. It would occur 7-14 days after ATG/ALG was given.<sup>9</sup> The main symptoms were pyrexia, rash, muscular soreness and anthralgia. Prednisolone could bring serum sickness reaction under control at the dose of 40-200 mg/d and larger dose was seldom used. No sequela was found. So ALG/ATG is safe in clinical practice.

The most important factor affecting the prognosis of the patients treated with ATG is relapse. In Europe, the reported rate was 30%-40%. The primary symptoms are red blood cell dependence and palette transfusion dependence, and CsA dependence recurring.<sup>6</sup> The long-term (10 years) relapse rate is 33%, with median relapse time of 570 day.<sup>10</sup> Sometimes, a few VSAA patients, staying in remittance for ten years after IST therapy, developed into clonal diseases such as PNH, MDS, AML and other tumors. The occurrence of PNH is 9%<sup>11</sup> and the 10-year cumulative incidence was 16%.<sup>12</sup> Another study reported that the incidence of clonal disease was 10%-40%.<sup>13-14</sup>

In our study, 25 VSAA patients were followed up for at least 1-year, no relapse was found. Only 1 case developed into PNH. Probably, the fewer relapse cases were due to shorter follow-up period. Longer follow-up period will still be conducted. In spite of this, the treatment of VSAA with ATG/ALG kidney-nourishing plus Chinese medicinal already showed promising efficacy. Moreover, up to date, no subjects have found dead. Further study will try to explore how VSAA exert its impact on complications.



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